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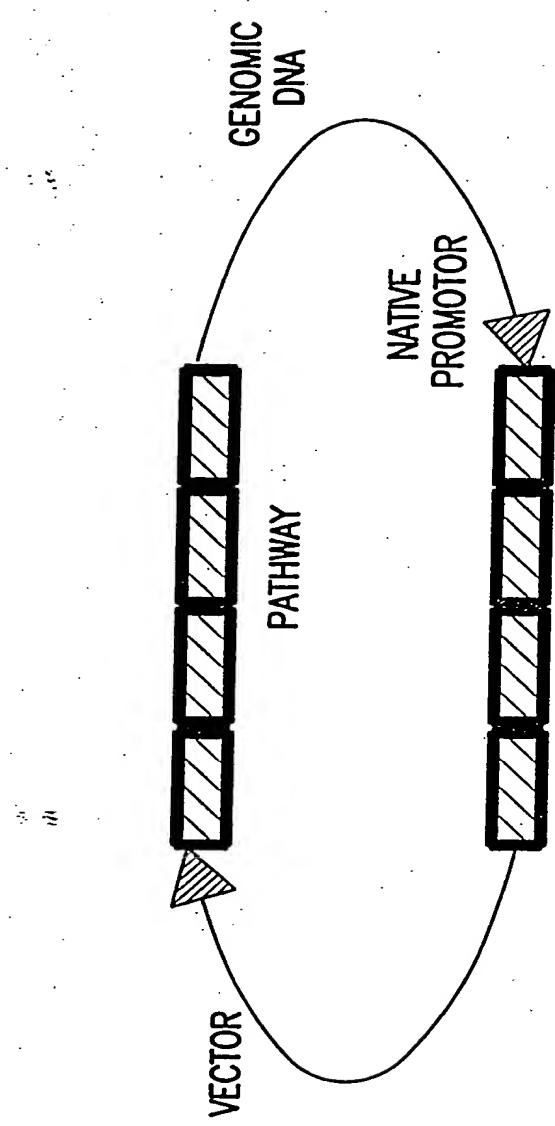


FIG. 1

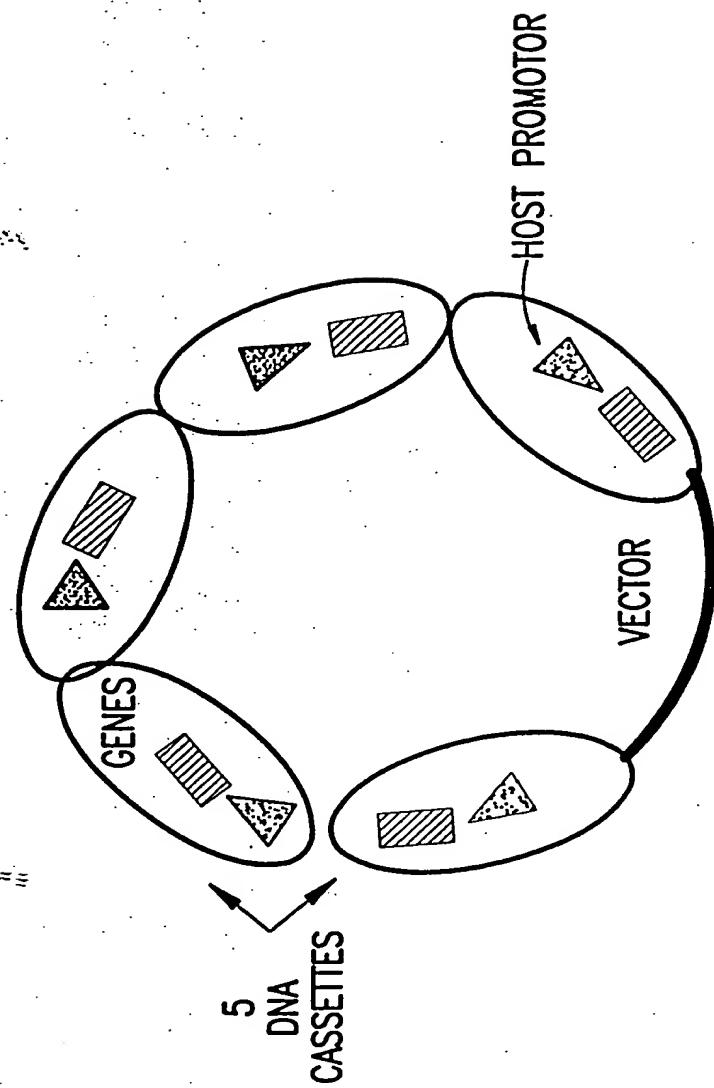


FIG.2

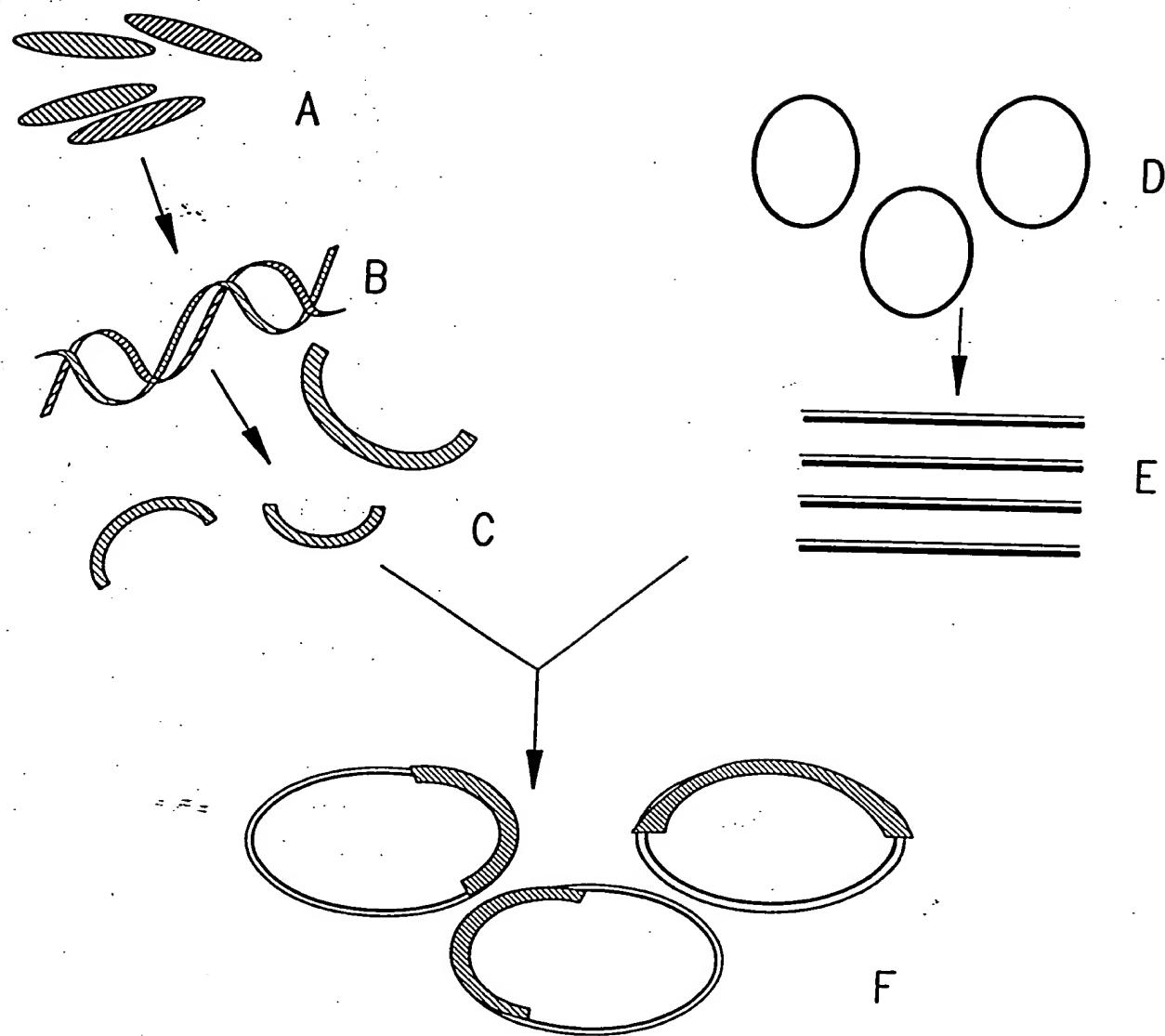


FIG.3

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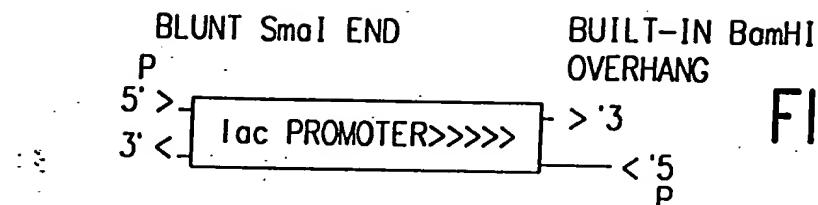


FIG.4A

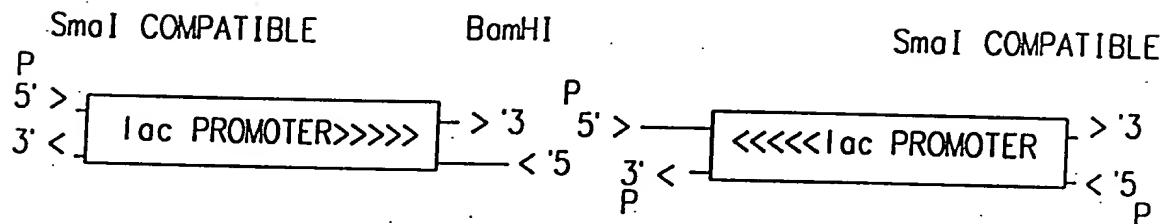


FIG.4B

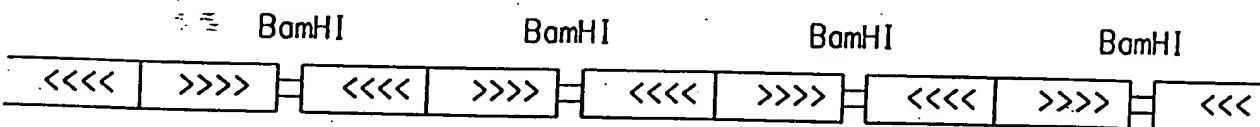


FIG.4C

PROMOTERS FOR
cDNA & gDNA INSERTS

5' - GAGTAGA[CT] . PCR PROMOTER .. CTCGAGGGGG-3'
3' - CTCATCTAGA[... FRAGMENT ...] GAGCTCGCCG-5'

Bgl II Xho I

CUT w/Bgl II & Xho I

^P
5' - GATCT[] . PCR PROMOTER .. C-3'
3' - A[...] FRAGMENT ... GAGCT-5'
Bgl II Xho I

FILL IN WITH dTTP & dCTP

^P
5' - GATCT[] . PCR PROMOTER .. CTC-3'
3' - A[...] FRAGMENT ... GAGCT-5'
Bgl II Xho I

TREAT w/ PHOSPHATASE

^P
5' - GATCT[] . PCR PROMOTER .. CTC-3'
3' - A[...] FRAGMENT ... GAGCT-5'
Bgl II Xho I

PROMOTORS READY TO
LIGATE TO INSERTS

TERMINATORS
FOR cDNA INSERTS

DISSIMILAR ENZYMES ON PROMOTER &
TERMINATOR FRAGMENTS ASSURE
DIRECTIONAL CLONING OF cDNA INSERTS,
(FOR EXAMPLE Xho I & Xma I)

ENZYME CLEAVAGE GENERATES
DEFINED ENDS, LEAVING
PROTECTED 3' BamHI SITE

KLENOW FILL IN OF
PROMOTERS & TERMINATORS
FRAGMENTS MAKE THEM INCAPABLE
OF INTER/INTRA LIGATION

PHOSPHATASE TREATMENT
GENERATES EQUAL STRENGTH
LIGATION PARTNERS

5' - GATCCCCGGG . PCR TERMINATOR . GGATCCCCGGG-3'
3' - CTAGGGCCC[...] FRAGMENT ... CCTAGGGCCC-5'
Xma I BamHI

CUT ONLY WITH Xma I

^P
5' - CGGGG . PCR TERMINATOR . GGATCCCCGGG-3'
3' - C[...] FRAGMENT ... CCTAGGGCCC-5'
Xma I BamHI

FILL IN WITH dCTP

^P
5' - CGGGG . PCR TERMINATOR . GGATCCCCGGG-3'
3' - CCC[...] FRAGMENT ... CCTAGGGCCC-5'
Xma I BamHI

^P
5' - CGGGG . PCR TERMINATOR . GGATCCCCGGG-3'
3' - CCC[...] FRAGMENT ... CCTAGGGCCC-5'
Xma I BamHI

TREAT w/ PHOSPHATASE

TERMINATORS READY TO
LIGATE TO cDNA INSERTS

FIG. 5A

PROMOTERS

5' - GAGTAGA[CTC] . PCR PROMOTER . [CTCGAGGGG-3'
 3' - CTCACTAGA ... FRAGMENT ...]GACCTGGG-5'
 Bgl II Xho I

CUT W/Bgl II & Xho I

P

5' - GATC[CTC] . PCR PROMOTER . [CTC-3'
 3' - A ... FRAGMENT ...]GAGCT-5'
 Bgl II Xho I

FILL IN WITH dTTP & dCTP

P

5' - GATC[CTC] . PCR PROMOTER . [CTC-3'
 3' - A ... FRAGMENT ...]GAGCT-5'
 Bgl II Xho I

TREAT W/ PHOSPHATASE

5' - GATC[CTC] . PCR PROMOTER . [CTC-3'
 3' - A ... FRAGMENT ...]GAGCT-5'
 Bgl II Xho I

PROMOTORS READY TO
LIGATE TO INSERTS

TERMINATORS

5' - CGGCCTCGAG . PCR TERMINATOR . GGATCCGGG-3'
 3' - GCGGGAGCTC ... FRAGMENT ... CCTAGGGCG-5'
 BamHI

CUT ONLY WITH Xho I

→

5' - TCGAG . PCR TERMINATOR . GGATCCGGG-3'
 3' - C ... FRAGMENT ... CCTAGGGCG-5'
 Xho I

FILL IN WITH dTTP & dCTP

P

5' - TCGAG . PCR TERMINATOR . GGATCCGGG-3'
 3' - CTC ... FRAGMENT ... CCTAGGGCG-5'
 Xho I

TREAT W/ PHOSPHATASE

KLENOW FILL IN OF
PROMOTORS & TERMINATORS
FRAGMENTS MAKE THEM INCAPABLE
OF INTER/INTRA LIGATION

PHOSPHATASE TREATMENT
CREATES EQUAL STRENGTH
LIGATION PARTNERS

TERMINATORS READY TO
LIGATE TO INSERTS

FIG. 5B

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FIRST STRAND cDNA SYNTHESIS PREPARED WITH Not I CONTAINING poly-dT PRIMER AND 5'-M_gCTP, AFTER 2nd STRAND SYNTHESIS, MODIFIED BamHI ADAPTERS ARE ADDED & cDNA IS DIGESTED WITH Not I, GIVING DIRECTIONAL cDNA GENE INSERTS

INSERT cDNAs

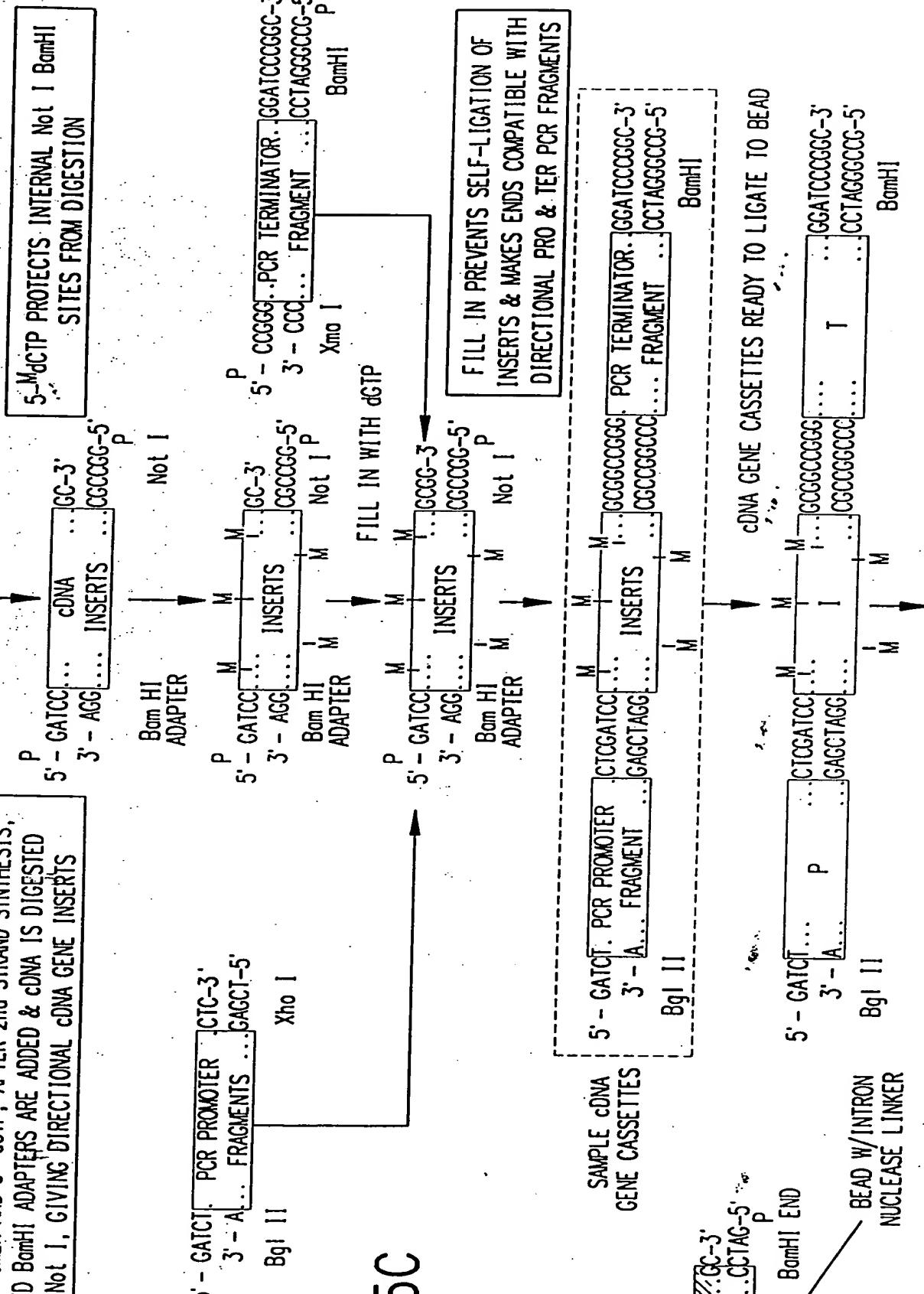


FIG. 5C

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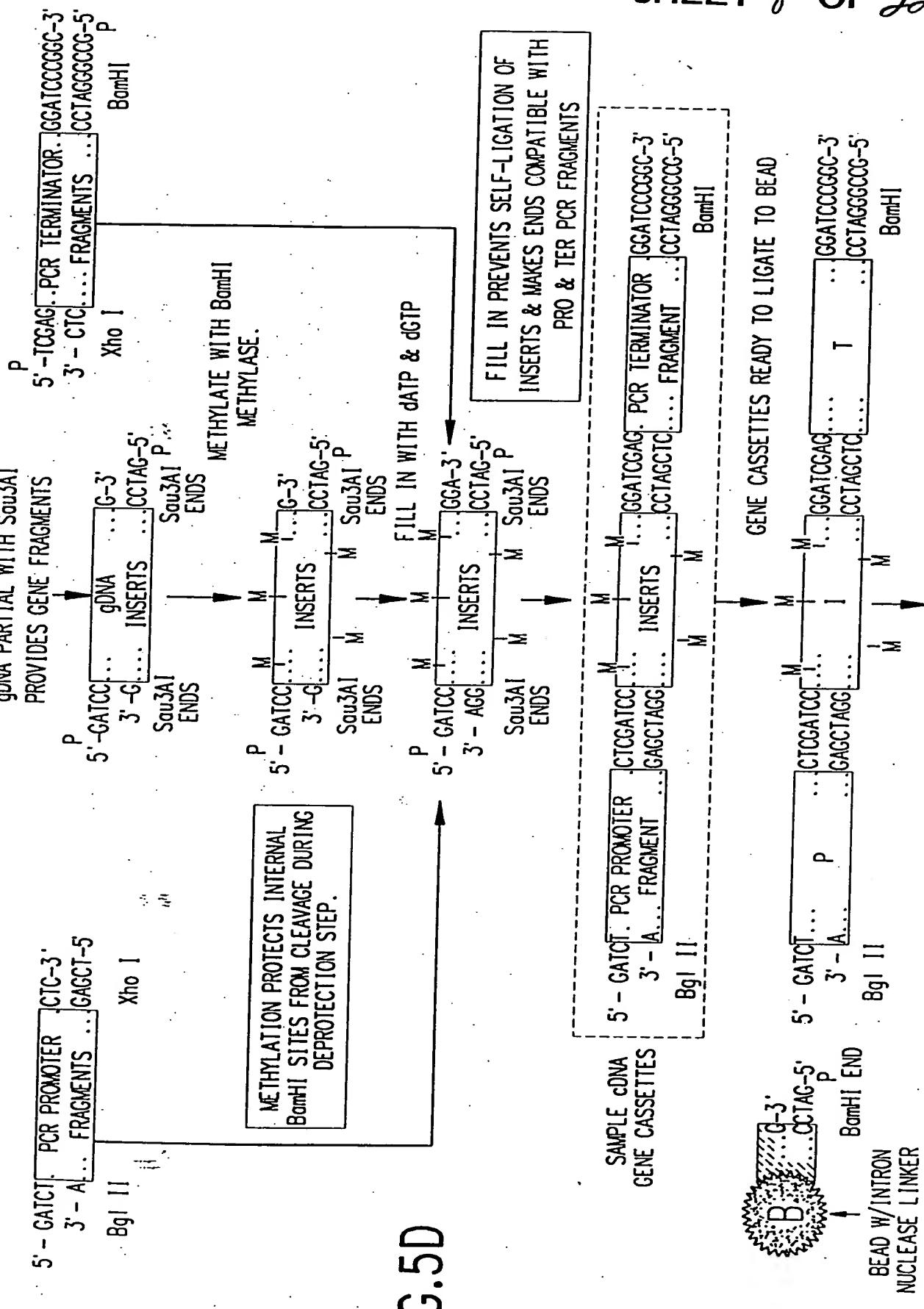
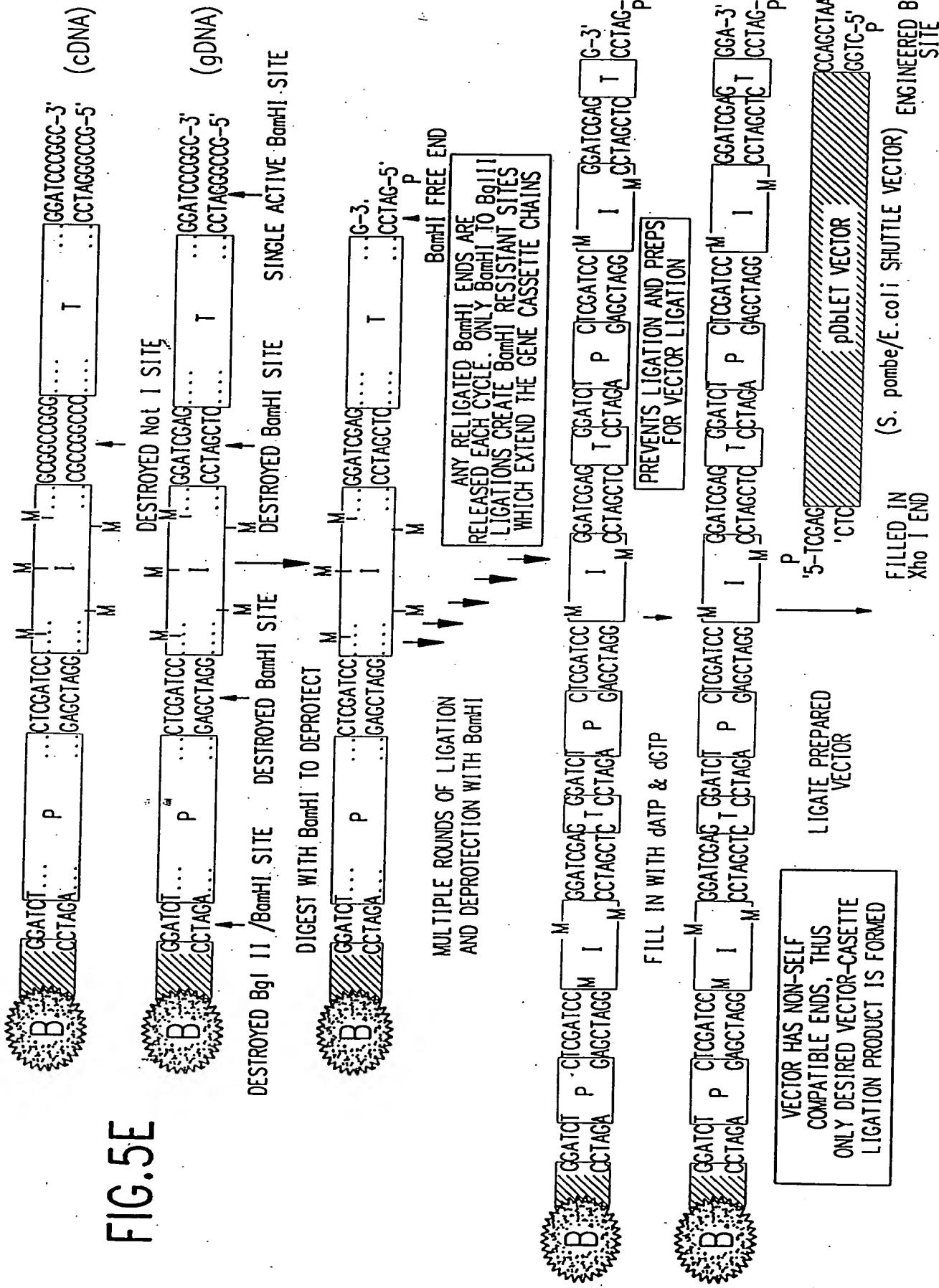


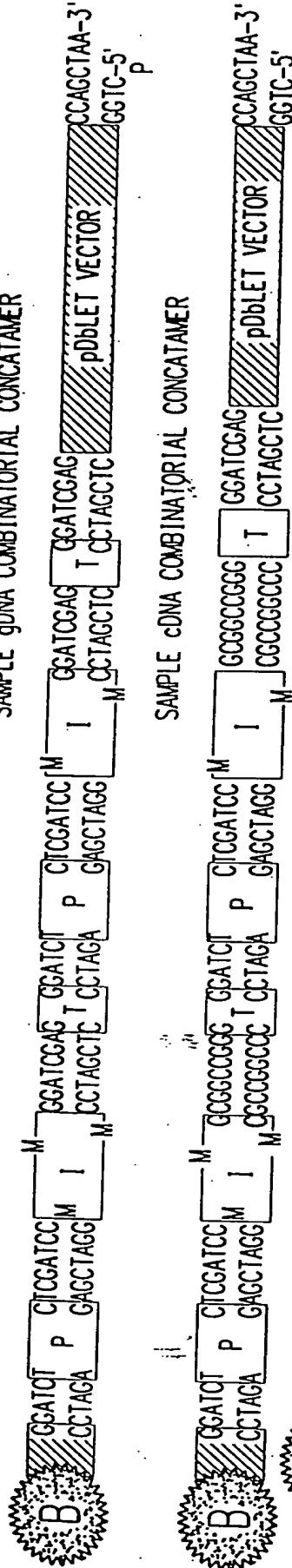
FIG. 5D



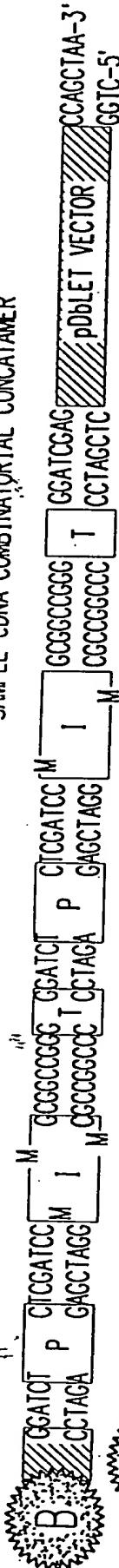
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SAMPLE gDNA COMBINATORIAL CONCATAMER



SAMPLE cDNA COMBINATORIAL CONCATAMER



SPUN AWAY & REMOVED

CUT WITH INTRON NUCLEASE TO RELEASE
SOLID-PHASE LIGATION BEAD
(SINGLE SITE IN LINKER OLIGO)

P
'5'-GGTAGCGAGGATCT-P-CCTGATCC-M-GGATCGAG-GGATCT-P-CCTGATCC-M-GGATCGAG-GGATCT-P-pBLLT VECTOR-CCAGCTAA-3'-GGTC-5'

BstXI COMPATIBLE
END

P
'5'-GGTAGCGAGGATCT-P-CCTGATCC-M-GGATCGAG-GGATCT-P-CCTGATCC-M-GGATCGAG-GGATCT-P-pBLLT VECTOR-CCAGCTAA-3'-GGTC-5'
BstXI
END

DILUTION FACILITIES
BstXI
END

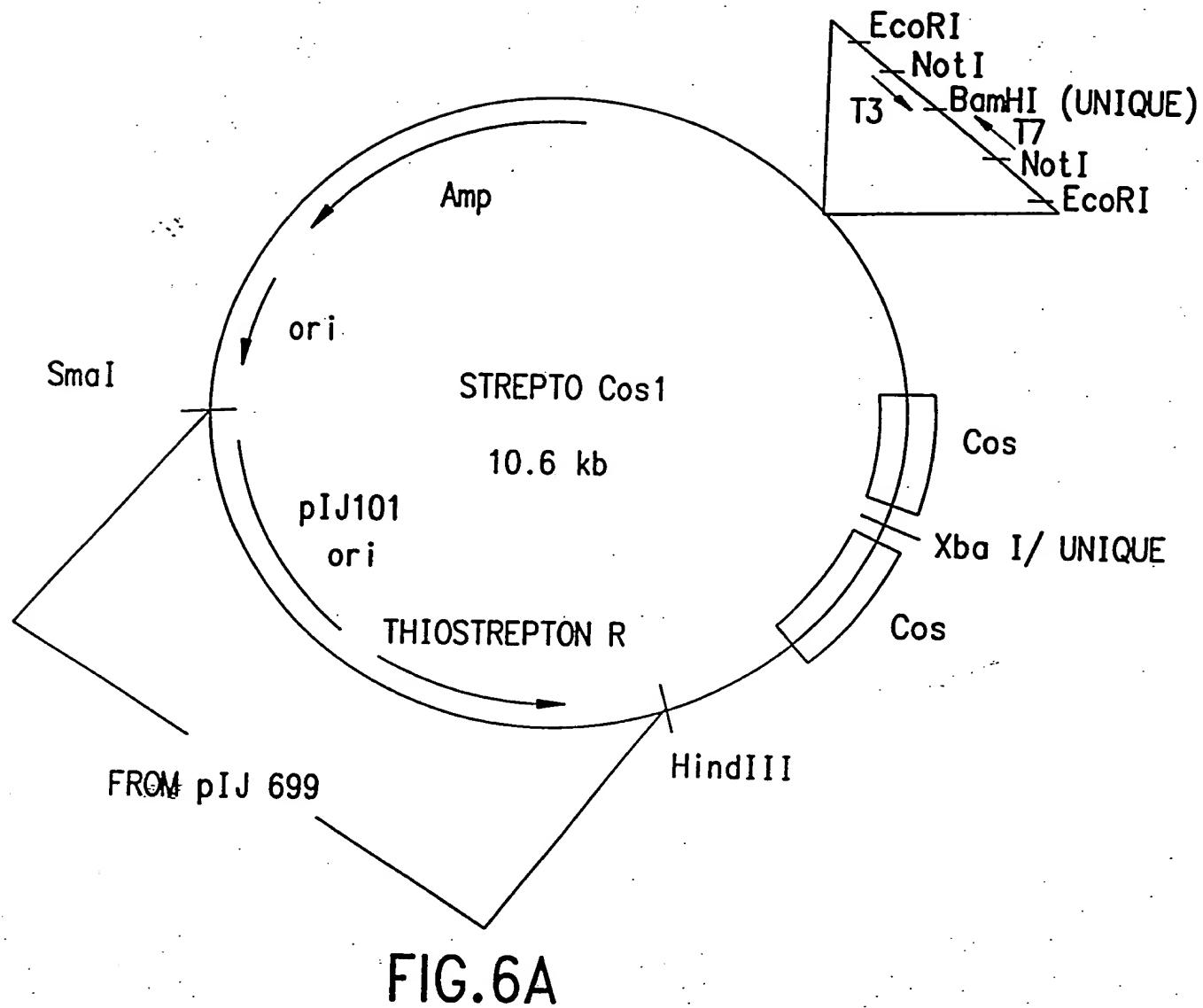
DILUTE, LIGATE
INTRA-MOLECULAR LIGATION
AND CIRCULARIZATION
BstXI
END

TRANSFORM S. pombe AND/OR E. coli AND
SCREEN RESULTING CLONES FOR
COMBINATORIAL ACTIVITIES

FIG. 5F

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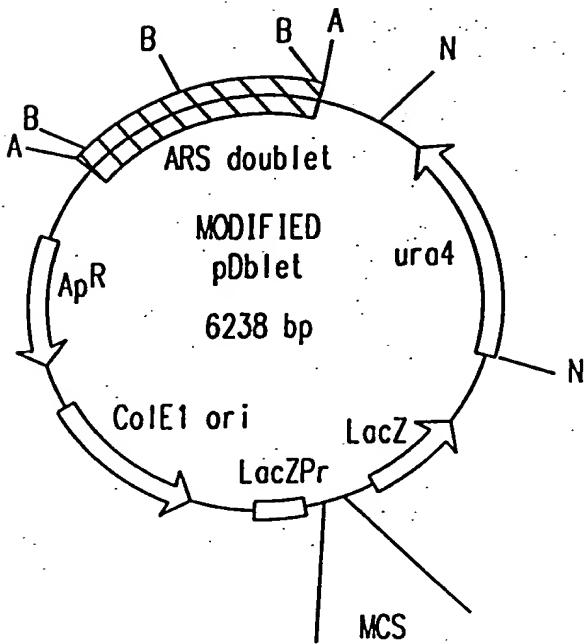
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MCS = SacI-NcoI-BstXI-NotI-Xba...

FIG.6B

5' CCTAGCCATGGCCACCTAACCTGGGATCGC 3'
3' TCGAGGATCGGTACCGGTGGATTGACCCTAGCCCCGG 5'
SacI NcoI BstXI NotI END

FIG.6C

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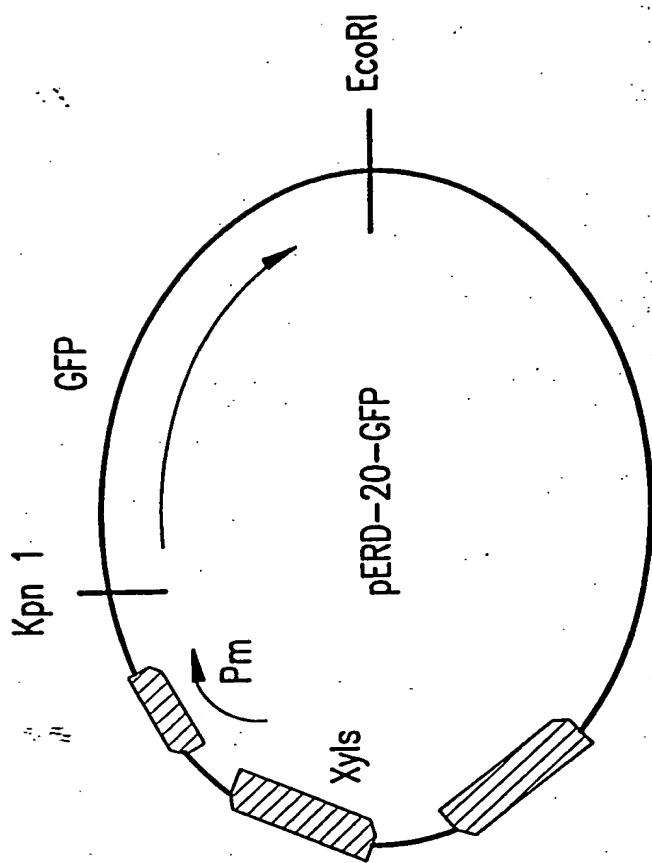


FIG. 7

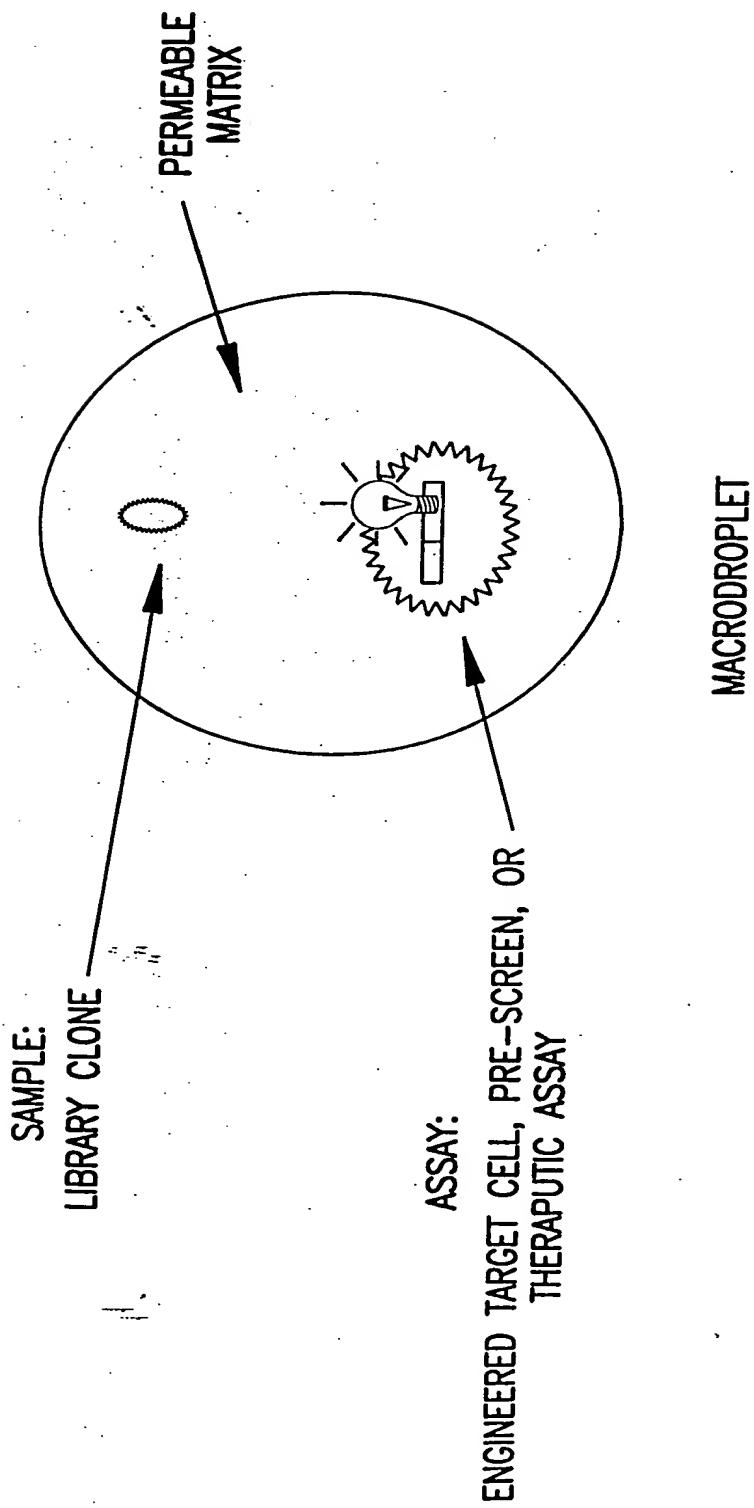


FIG.8

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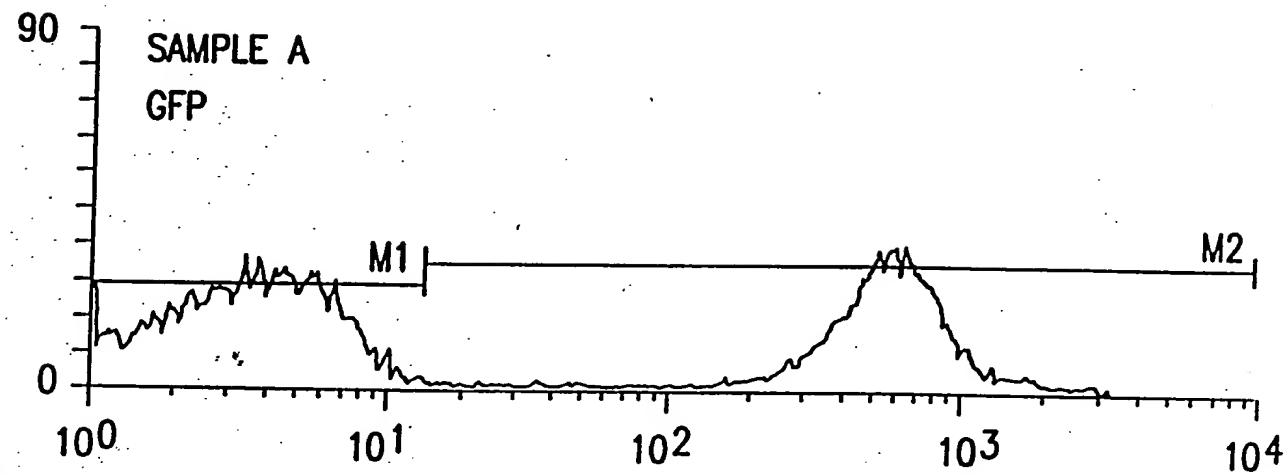


FIG.9A

02986786 - 120597

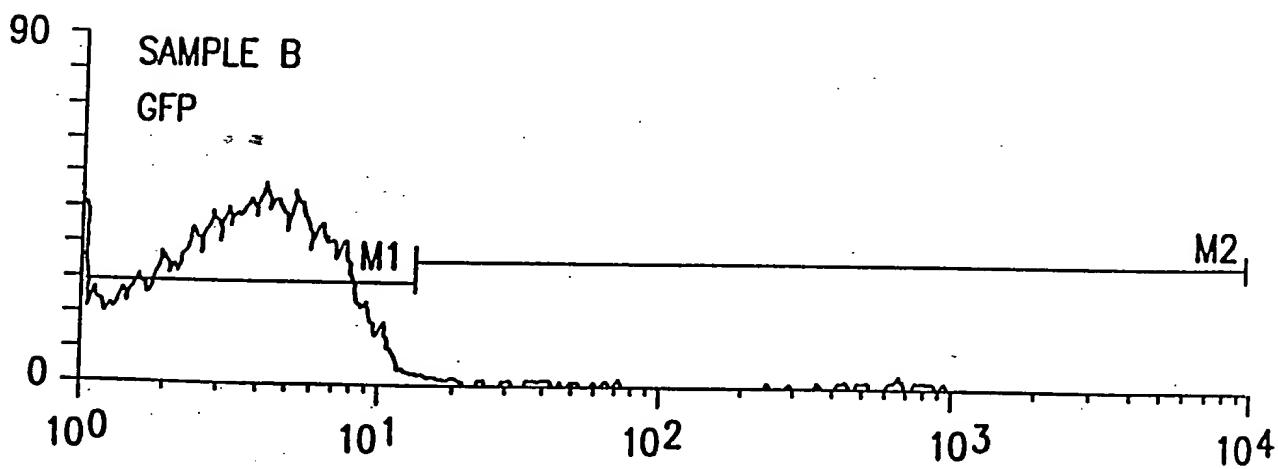


FIG.9B

FIG. 10

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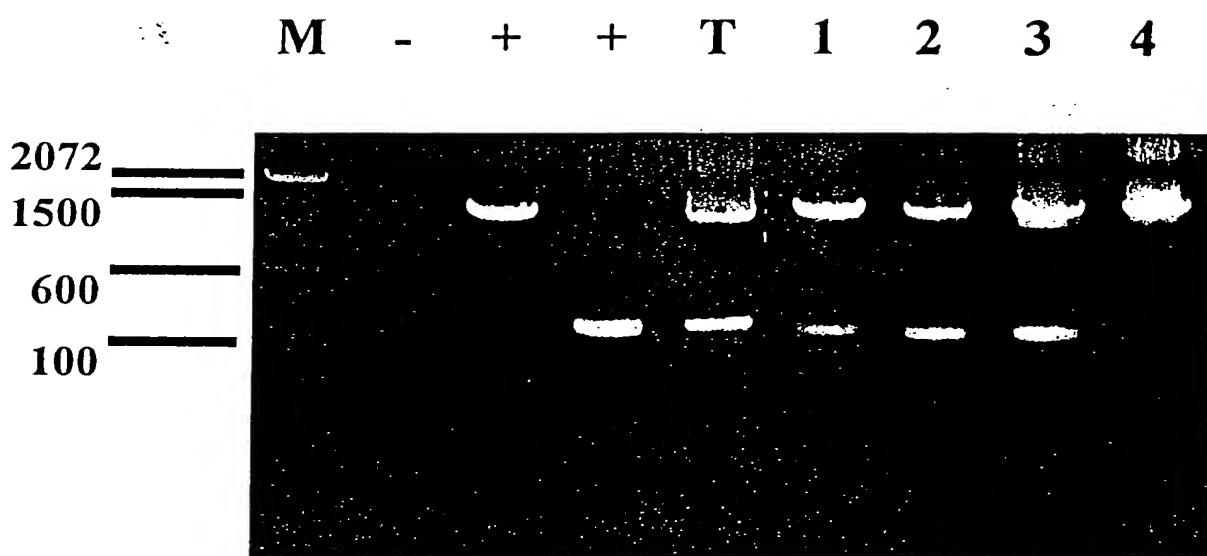


FIG. 11

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POOL 1

M - + + 1 2 3 4 5 6 7 8 9 10

2072
1500
600
100

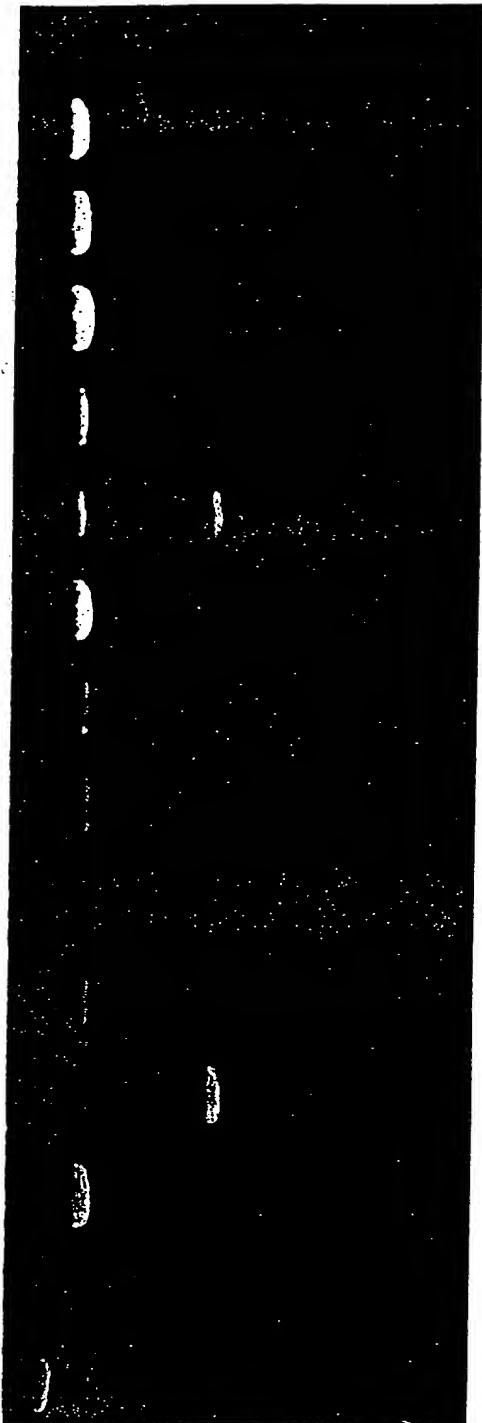


FIG.12A

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POOL 2

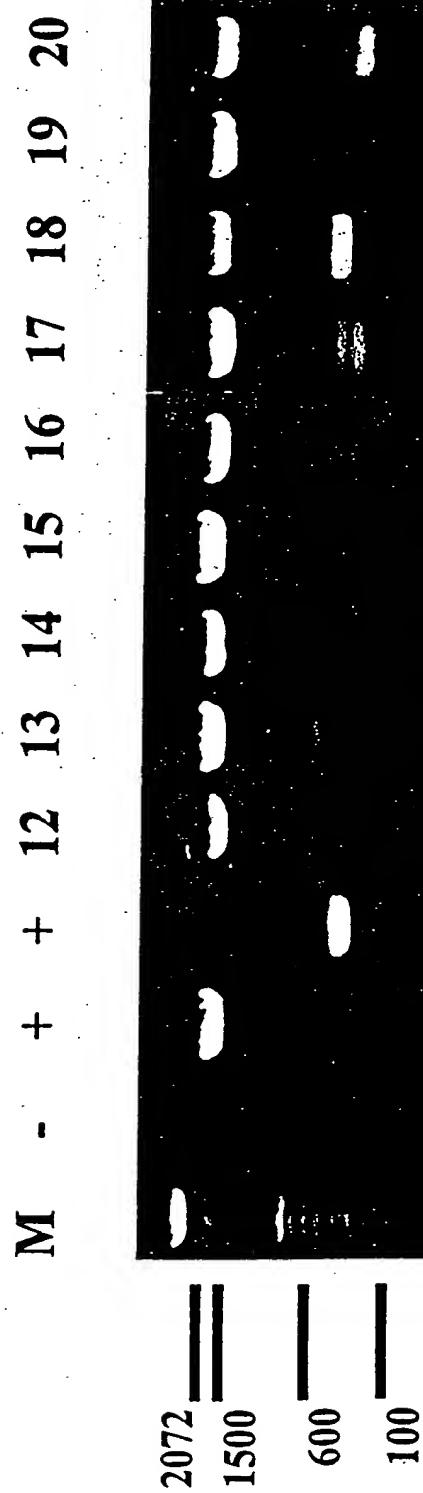


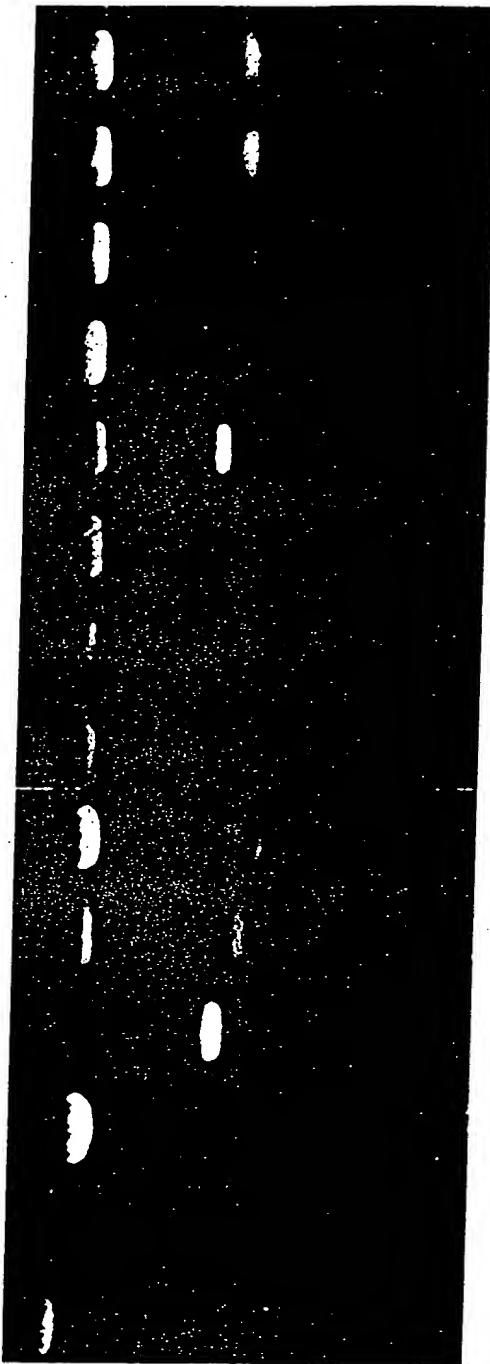
FIG.12B

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POOL 3

M - + + 21 22 23 27 30 31 32 33 34 35



2072
1500
600
100

FIG. 12C

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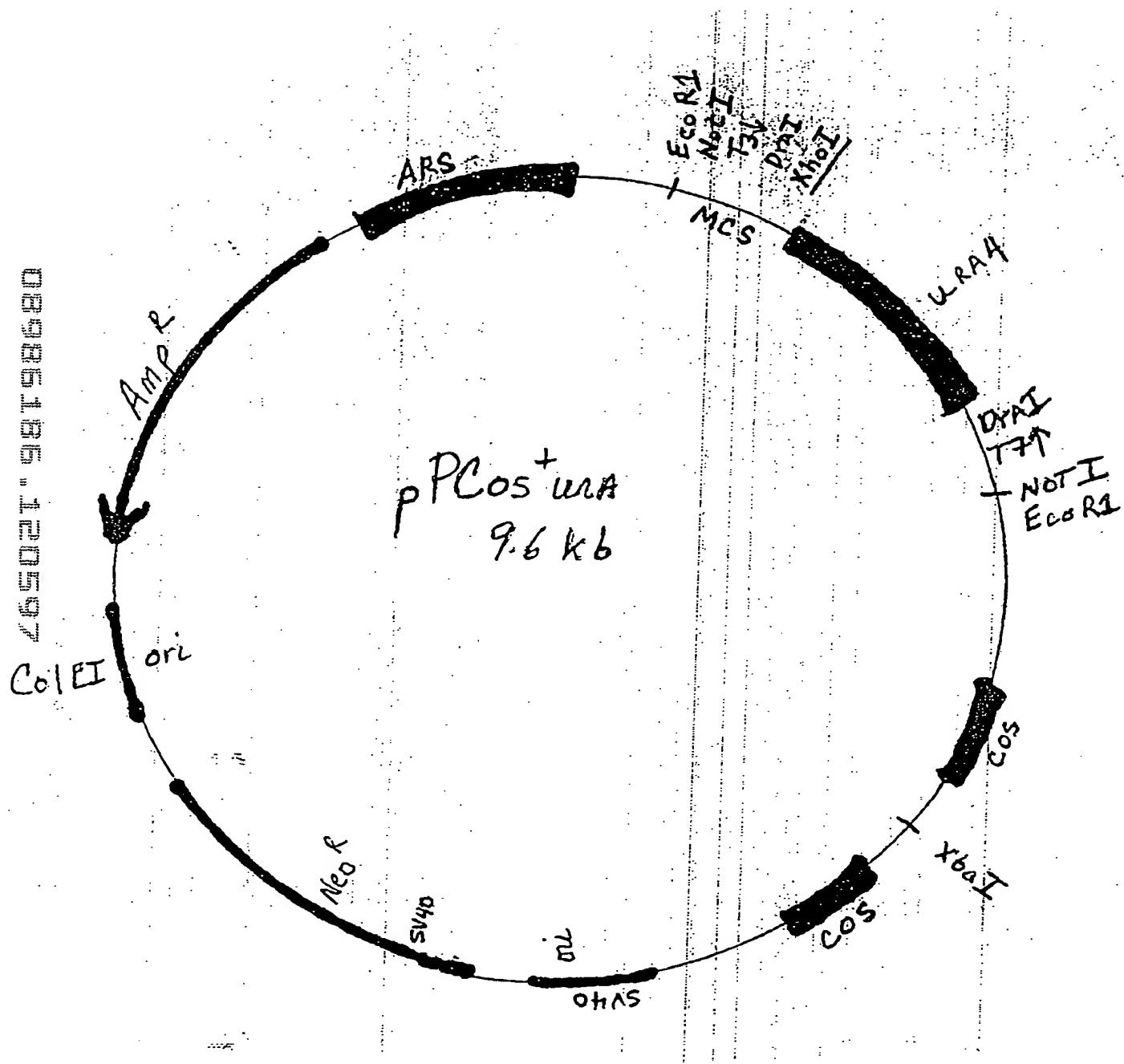


FIG. 13

085861866592

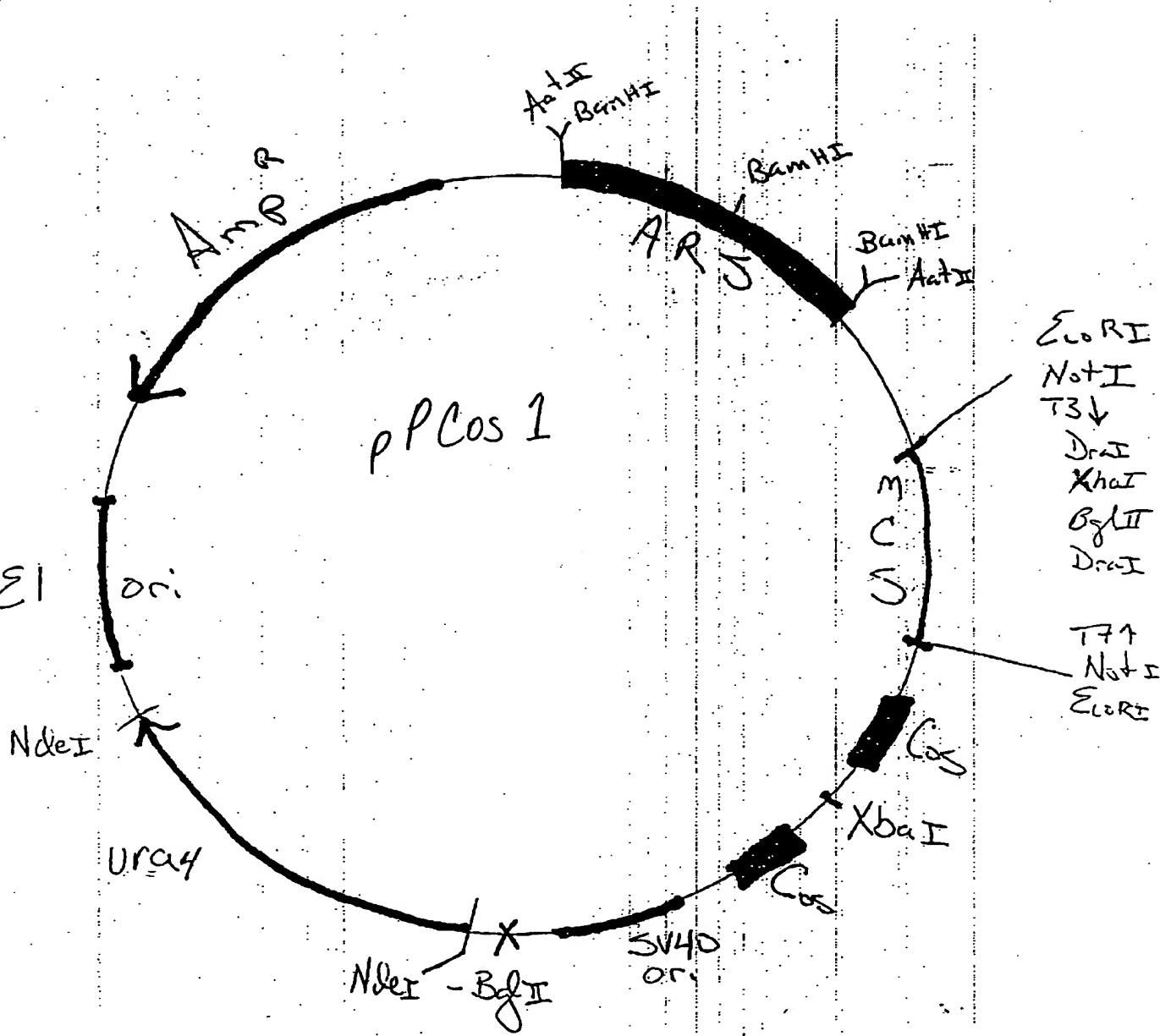


FIG. 14